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International Study of 'Artemia' VIII Comparison of the Chlorinated Hydrocarbons and Heavy Metals in Five Different Strains of Newly Hatched 'Artemia' and a Laboratory-Reared Marine Fish

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International Study on *Artemia*[1] VIII. Comparison of the chlorinated hydrocarbons and heavy metals in five different strains of newly hatched *Artemia* and a laboratory-reared marine fish

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Abstract

Newly hatched nauplii of *Artemia* from Brazil, Australia, Italy, and the United States (Utah and San Pablo Bay, California) were analyzed for chlorinated hydrocarbons. The Brazil and Australia nauplii contained very low levels of PCB and chlorinated insecticides. Italian nauplii contained the highest levels of HCB, BHCs and DDTs, while San Pablo nauplii were highest in chlordanes, dieldrin and PCBs. With the exception of 188 ppb pp-DDT in Italy nauplii, none of the residues exceeded 100 ppb on a wet weight basis.

Atlantic silversides (*Menidia menidia*) and mud crabs (*Rhithropanopeus harrisii*) from two *Artemia* feeding studies were analyzed. Residue levels in the organisms generally reflected the concentrations in the nauplii.

Attempts to correlate nauplii residue levels with the survival of laboratory-reared marine fish (Atlantic silversides and winter flounder *Pseudopleuronectes americanus*) and crabs (mud crab and rock crab *Cancer irroratus*) disclose no obvious component(s) which could totally account for the poorer performance of the Utah and San Pablo strains.

The satisfactory performance of the Italian strain seems to eliminate HCB, BHCs and DDTs as causative agents *per se*. If chlorinated hydrocarbons are involved with the poorer survival of marine fish and crabs fed San Pablo nauplii, chlordane, dieldrin or high molecular weight PCBs would appear to be the most likely suspects.

Twelve metals, including copper, lead, and cadmium, were measured by atomic absorption and neutron activation analysis. Differences between nauplii were small and no particularly high concentrations were observed.

Introduction

In 1970, Bookhout and Costlow reported that *Artemia* nauplii from salt pools near San Francisco, California supported better survival of four crab larval species than nauplii from

[1] International Interdisciplinary Study on *Artemia* Strains coordinated by the Artemia Reference Center, State University of Ghent, Belgium.

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16. ABSTRACT

Newly hatched nauplii of *Artemia* from Brazil, Australia, Italy, and the United States (Utah and San Pablo Bay, California) were analyzed for chlorinated hydrocarbons. The Brazil and Australia nauplii contained very low levels of PCB and chlorinated insecticides, Italian nauplii contained the highest levels of HCB, BHCs and DDTs, while San Pablo nauplii were highest in chlordanes, dieldrin and PCBs. With the exception of 188 ppb pp-DDT in Italy nauplii, none of the residues exceeded 100 ppb on a wet weight basis.

Atlantic silversides (*Menidia menidia*) and mud crabs (*Rhithropanopeus harrisii*) from two *Artemia* feeding studies were analyzed. Residue levels in the organisms generally reflected the concentrations in the nauplii.

Attempts to correlate nauplii residue levels with the survival of laboratory-reared marine fish (Atlantic silversides and winter flounder *Pseudopleuronectes americanus*) and crabs (mud crab and rock crab *Cancer irroratus*) disclose no obvious component(s) which could totally account for the poorer performance of the Utah and San Pablo strains.

The satisfactory performance of the Italian strain seems to eliminate HCB, BHCs and DDTs as causative agents *per se*. If chlorinated hydrocarbons are involved with the poorer survival of marine fish and crabs fed San Pablo nauplii, chlordane, dieldrin or high molecular weight PCBs would appear to be the most likely suspects.

Twelve metals, including copper, lead, and cadmium, were measured by atomic absorption and neutron activation analysis. Differences between nauplii were small and no particularly high concentrations were observed.

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the Great Salt Lake in Utah, and suggested that differences in performance might be due to different DDT levels in the nauplii (7.5 ppm *versus* 2.3 ppm, wet weight basis). Two years later, Wickins (1972) fed newly hatched *Artemia* from Utah and San Francisco to prawn larvae and again found that Utah nauplii did not support growth to the post larval stage. DDT levels in *Artemia* eggs and nauplii were considerably lower than those reported by Bookhout and Costlow (1970) (< 0.8 ppm for cysts and < 0.2 ppm for nauplii, dry weight basis). He also reported measurable quantities of PCBs (0.04-0.24 ppm). Analysis of eggs and nauplii for several metals revealed detectable amounts of cadmium (0.87-1.15 ppm, dry weight), zinc (51-66 ppm), and copper (4.4-27.5 ppm) in eggs and nauplii. PCB, cadmium and copper levels were somewhat higher in the Utah samples, but "none were confidently labeled as the cause of the poor food value of the Utah nauplii".

As part of a cooperative effort (International Study on *Artemia*) to characterize and compare *Artemia* cysts from different geographical locations, we have analyzed freshly hatched nauplii and two marine organisms fed the nauplii for residues of chlorinated hydrocarbon insecticides and PCBs. We have also analyzed cysts and nauplii from the five locations for heavy metals and the results are reported here.

Methods and materials

Materials

Dehydrated cysts from five geographical locations were provided by the Artemia Reference Center (Ghent, Belgium). The origins of the cysts were Macau, Brazil (Companhia Industrial do Rio Grande do Norte, CIRNE-Brand, harvested 1978); Shark Bay, Australia (World Ocean, lot no. 113); Margherita di Savoia, Italy (harvested 1977); Great Salt Lake, Utah, USA (harvested 1977); and San Pablo Bay, California, USA (Living World, San Francisco Bay Brand, Inc., lot no. 1628). In addition, two 1975 lots of San Francisco Bay *Artemia* (San Francisco Bay Brand, Inc., lot no. 313/3006 and lot no. 321995) were analyzed and were used in biological tests at the Narragansett EPA laboratory.

Artemia nauplii were hatched as described by Beck *et al.* (1980).

Samples of *Menidia menidia* were obtained from experiment I of Beck *et al.* (1980). Newly hatched larvae were fed San Pablo Bay nauplii for 10 days. The fish (*circa* 2.4 mg) were then fed newly hatched nauplii from the five geographical locations for 18 days. At termination, the fish weighed approximately 30 mg.

Newly hatched (stage I) zoeal *Rhithropanopeus harrisii* larvae were fed *Artemia* nauplii from four of the geographical locations for 9 days and sacrificed before the stage IV megalopa molt (Johns *et al.*, 1980).

All biological samples were stored at -20 °C until analysis.

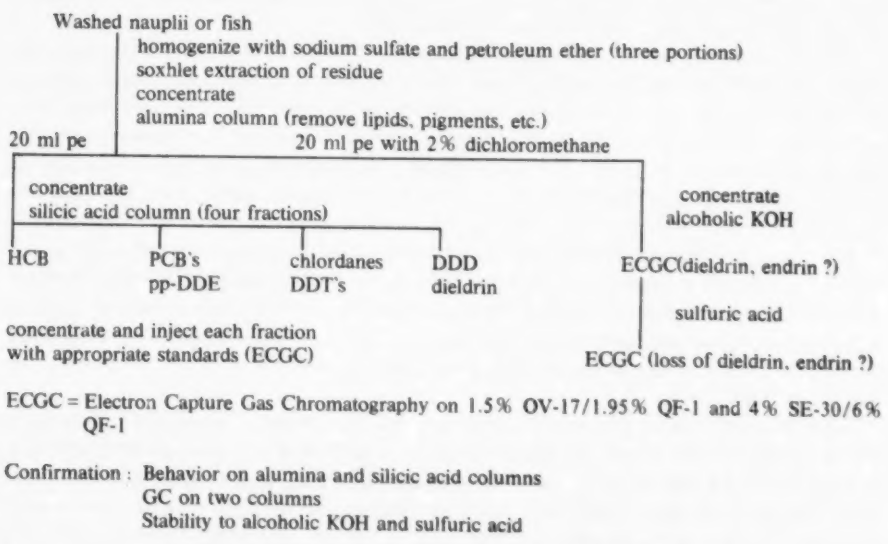
Chlorinated hydrocarbon analysis

The analytical scheme for chlorinated hydrocarbons in fish and *Artemia* nauplii is summarized in Fig. 1. Frozen samples were thawed and representative subsamples weighed. The samples were blended with anhydrous sodium sulfate and three successive portions of petroleum ether followed by extraction in a Soxhlet apparatus and concentration by Kuderna-Danish evaporative concentrator. The extracts were chromatographed on Woelm alumina,

activity grade III, to remove lipids, pigments and other co-extractives. Two fractions were collected : the first, containing PCBs and the majority of insecticides, was further fractionated on silicic acid (Bidleman *et al.*, 1978), primarily to separate PCBs from other components. The second fraction from alumina was checked for dieldrin and endrin before and after treatment with alcoholic potassium hydroxide, in which dieldrin and endrin are stable, and concentrated sulfuric acid, in which they are not. Sulfuric acid and, less frequently, alcoholic potassium hydroxide were also employed for pesticide confirmation and clean-up of the third and fourth silicic acid fractions.

The crab samples were extracted and cleaned-up by a slightly different procedure. Small samples (0.2-0.4 g wet weight) were ground with sodium sulfate and dichloromethane in a Potter Elvehjem tissue homogenizer and chromatographed on florisil instead of alumina to obtain better separation of dieldrin and endrin from other chlorinated hydrocarbons.

Individual column fractions were analyzed by dual column electron capture gas chromatography (Tracor MT-220 gas chromatograph, Ni-63 detectors, 180×0.4 cm glass columns packed with 1.5% OV-17/1.95% QF-1 or 4% SE-30/6% QF-1 on 100/120 mesh Supelcon AW-DCMS). Peaks were identified by retention time and quantitated using peak heights as compared with standards. The residues reported have been corrected for reagent blanks which were run with each set of five samples. Nauplii values represent duplicate analyses of 2-6 g samples, *Menidia* values are from a single analysis of 1-2 g samples, while the mud crab data are from a single analysis of 0.2-0.4 g samples.



ECGC = Electron Capture Gas Chromatography on 1.5% OV-17/1.95% QF-1 and 4% SE-30/6% QF-1

Confirmation : Behavior on alumina and silicic acid columns
GC on two columns
Stability to alcoholic KOH and sulfuric acid

FIG. 1. Flow chart of chlorinated hydrocarbon analysis

Metal analysis

Screening for metals in washed cysts and freshly hatched nauplii was accomplished by atomic absorption spectrometry and neutron activation analysis. Freeze-dried samples were digested with nitric and perchloric acids, filtered and diluted to 25 or 100 ml for analysis by flame atomic absorption (Fe, Zn, Pb, Cu, Cr, Cd, and Ni). For neutron activation, freeze-dried samples were sealed in polyethylene vials and irradiated, along with appropriate standards, for 7 hr with thermal neutrons (flux approximately 4×10^{12} neutrons/cm^2/sec) at the Rhode Island Nuclear Science Center reactor. After a 21-day cooling period, gamma spectra were measured with a Ge(Li) detector and 4096 channel pulse height analyzer (Fe, Zn, Cr, Co, Sc, Sb, Se, and Rb).

Results

The chlorinated hydrocarbon contents of nauplii of *Artemia* from the five locations are presented in Table I, while the results of five analyses of nauplii from one lot of cysts are shown in Table II to illustrate the variation encountered. In Table II, the coefficients of variation for individual compounds vary from 16 to 85%, with the majority less than 40%. The five samples were analyzed over a 6-month period and therefore reflect biological as well as analytical variation. Because of the manner in which they are formed, collected, pooled and processed, it appears to us that cysts within a given lot might well contain variable amounts of environmental contaminants. For comparing nauplii from different locations (Table I) the San Pablo Bay values are based on the first two replicates listed in Table II, which turn out to have means very close to the means for five determinations.

It can be seen that there are differences between nauplii from cysts from different locations. In general, the Brazil and Australia samples were very low in all chlorinated hydrocarbons. The Italian nauplii contained higher amounts of HCB (hexachlorobenzene), total BHC (hexachlorocyclohexane) and DDTs than nauplii from other locations. The Italian sample was also second highest in PCBs. San Pablo Bay nauplii were intermediate in DDTs and highest in chlordanes, dieldrin and PCBs. One San Francisco 1975 sample seems to resemble the San Pablo Bay sample, while the other 1975 lot has lower levels of almost every component.

A comparison of the chlorinated hydrocarbon levels in fish fed nauplii from five locations is shown in Table III. Chlordane levels were higher in the Italian and San Pablo Bay samples, while dieldrin was highest in the fish fed Australian and San Pablo Bay nauplii. The DDTs were highest in the Italian sample, and San Pablo-fed fish also reflected the DDT present in the nauplii. PCB levels in fish appear to reflect nauplii levels, with Italian and San Pablo samples highest in PCBs.

Table IV shows the residue levels in mud crab fed four strains of *Artemia*. Unfortunately Italian nauplii were not fed in the experiment from which these crab samples were collected. Because of the limited amount of material available for analysis, only single determinations were possible for *Menidia* and *Rhithropanopeus*. The mud crab values must be viewed with additional caution since sample size was only 0.2-0.4 g. The 0-day column gives the chlorinated hydrocarbon content of a sample of crab larvae taken at the start of the feeding period. With a few exceptions, the 9-day crabs contained more residues than any of the 0-day crabs. However, the bioaccumulation of the dietary pesticides by the crabs was less than that

TABLE I

Chlorinated hydrocarbons in *Artemia* nauplii (ng/g wet weight)[1]

	Brazil	Australia	Italy	Utah	San Pablo Bay	SFB-75a (313)	SFB-75b (321)
HCB	0.1	0.2	4.4	0.4	0.5	0.5	0.7
α-BHC	1.1	0.6	5.2	5.9	3.1	2.8	1.4
γ-BHC	0.8	–	5.0	1.9	3.2	1.0	2.8
c-chlordane	0.1	–	2.2	0.8	14.	1.3	6.6
t-nonachlor	0.03	–	–	1.2	5.5	1.4	8.3
dieldrin	0.2	0.1	–	0.7	1.4	0.2	1.2
op-DDE	0.4	–	3.9	0.7	5.3	0.9	6.7
pp-DDE	1.2	1.3	100.	2.2	18.	2.0	10.
pp-DDD	0.4	–	74.	3.0	13.	3.8	22.
op-DDT	0.4	0.2	60.	0.2	1.0	–	2.0
pp-DDT	1.9	4.3	188.	1.2	4.6	1.9	2.0
Σ DDT's	4.3	5.8	422.	7.3	42.	8.7	43.
PCB (1016)	5.3	2.0	5.3	3.2	14.	6.4	18.
PCB (1254)	1.6	0.9	19.	6.5	29.	3.5	23.
PCB (1260)	–	–	7.5	4.9	22.	3.5	2.8
Σ PCB's	6.9	2.9	32.	15.	66.	13.	43.

[1] Two determinations.

TABLE II

Chlorinated hydrocarbons in San Pablo Bay *Artemia* nauplii.
Results of five analyses (ng/g wet weight)

	1	2	3	4	5	$\bar{x}$ s^1
Sample wt (g)	3.13	2.04	6.07	2.00	2.00	
HCB	0.67	0.41	0.58	0.34	0.39	0.48 ± 0.14
α-BHC	3.73	2.45	3.15	1.15	2.19	2.53 ± 0.98
γ-BHC	4.19	2.24	2.48	1.18	4.74	2.97 ± 1.47
c-chlordane	20.4	7.50	5.86	6.42	11.9	10.42 ± 6.06
t-nonachlor	9.27	1.72	14.4	2.88	3.32	6.32 ± 5.38
dieldrin	NA	2.15	1.88	0.65	1.01	1.42 ± 0.70
op-DDE	8.35	2.18	6.45	3.32	6.75	5.41 ± 2.57
pp-DDE	22.6	12.8	19.1	14.1	16.5	17.0 ± 3.94
pp-DDD	13.6	12.0	14.9	9.75	14.7	13.0 ± 2.15
op-DDT	1.53	0.46	1.51	3.03	3.95	2.10 ± 1.38
pp-DDT	8.51	0.79	6.35	2.88	5.00	4.71 ± 3.00
PCB (1016)	17.2	10.1	8.80	12.4	7.90	11.3 ± 3.72
PCB (1254)	38.0	20.9	17.7	30.5	38.4	29.1 ± 9.55
PCB (1260)	27.8	17.1	36.6	20.8	11.2	22.7 ± 9.83

[1] Standard deviation $s = \left[\frac{\Sigma(x-\bar{x})^2}{n-1}\right]^{1/2}$

TABLE III

Chlorinated hydrocarbons in Atlantic silversides *Menidia menidia* fed freshly hatched nauplii of *Artemia* from five locations (ng/g wet weight)[1]

	Brazil	Australia	Italy	Utah	San Pablo
HCB	0.2	–	1.2	0.2	0.7
α-BHC	1.5	2.7	1.2	1.1	3.0
γ-BHC	0.4	–	0.6	0.4	0.8
c-chlordane	1.7	–	8.0	2.1	7.1
t-nonachlor	1.8	–	–	0.9	34.
oxychlordane	–	–	–	–	1.9
dieldrin	1.6	3.9	1.9	1.5	7.5
op-DDE	–	–	3.0	–	13.
pp-DDE	3.8	5.7	178.	5.8	55.
pp-DDD	2.2	2.5	46.	19.	27.
op-DDT	–	0.2	20.	1.5	1.3
pp-DDT	1.5	3.0	360.	2.8	13.
PCB (1016)	11.	17.	21.	16.	62.
PCB (1254)	19.	22.	91.	30.	66.
PCB (1260)	4.7	4.5	17.	7.0	122.
% survival[2]	87	87	80	80	58

[1] Single analysis. Samples from experiment I (Beck *et al.*, 1980).

[2] Beck *et al.* (1980).

TABLE IV

Chlorinated hydrocarbons in mud crab (*Rhithropanopeus harrisii*) fed freshly hatched nauplii of *Artemia* from four locations (ng/g wet weight)[1]

	0-day	Brazil	Australia	Utah	San Pablo
HCB	0.1	0.7	–	1.2	0.6
α-BHC	0.8	1.2	5.2	2.3	1.3
γ-BHC	–	0.2	1.8	0.6	0.3
c-chlordane	0.3	0.1	1.3	0.9	3.5
t-nonachlor	–	–	–	–	–
oxychlordane	0.9	0.3	0.7	0.6	3.4
dieldrin	0.8	–	–	0.8	1.8
op-DDE	–	–	–	–	6.3
pp-DDE	2.2	2.4	6.1	4.0	19.
pp-DDD	–	–	–	2.1	6.5
op-DDT	–	–	1.5	–	–
pp-DDT	0.3	1.0	2.4	1.1	1.8
PCB (1016)	4.0	21.	29.	30.	16.
PCB (1254)	10.	14.	20.	20.	33.
PCB (1260)	2.5	3.2	2.0	6.1	25.
Survival	75	77	61	0[2]	0[2]

[1] Single analysis. No crab sample available for Italy.

[2] Larvae did not survive beyond megalopa stage (Johns *et al.*, 1980).

found in silversides. Major differences between the crabs fed various geographical strains were the higher levels of BHC in the Australian treatment and chlordane, DDT and PCB 1260 in the San Pablo Bay treatment.

The results of the metal analyses are shown in Table V. Because of variations in blanks and sample size, we have not attempted to insert values for minimum detectable levels, but only report positive values after correcting for blanks. All cysts were high in iron, much of it associated with the chorion. Zinc was the second most abundant element. The Utah cysts contained three times more copper than any other cyst, but the nauplii copper concentration was lower and similar to that in the nauplii from Brazil, Italy, and San Pablo Bay. This is true for most of the metals ; nauplii levels are lower than cyst levels and generally show less variation between strains.

TABLE V

Metals in cysts and nauplii of *Artemia* from five locations (μg/g dry weight)

	Brazil		Australia	Italy		Utah		San Pablo Bay	
	c[1]	n[1]	c[2]	c	n	c	n	c	n
Fe	804.	62.	820.	1 860.	70.	800.	47.	1 380.	46.
Zn	59.	89.	144.	125.	104.	81.	102.	78.	98.
Pb	8.9		2.1	17.	3.0	5.1	6.2	6.6	3.8
Cu	4.7	6.3	4.6	14.	9.2	42.	8.2	10.2	10.8
Cd	0.02	0.15	0.15	0.09	0.12	0.28	0.14	0.26	0.10
Cr		1.4	1.8	3.7	0.66	2.0	1.1	4.4	0.48
Ni	0.23	0.29	0.50	4.0	0.09	2.9	0.70	6.6	0.12
Co	0.35	0.38	0.20	1.6	0.14	0.34		1.9	
Sc	0.02		0.01	0.41		0.03		0.31	
Sb	0.53		0.53						
Sc	1.4		1.2	1.2	1.0	1.5	2.1	2.3	0.83
Rb	6.5	12.			7.5	23.	23.	9.1	6.5

[1] c = cyst ; n = nauplii.

[2] Australian nauplii not analyzed (insufficient sample).

Nauplii concentrations, which should give a better indication of potential problems in using decapsulated cysts or nauplii as food for other organisms, range from none detected (ND) to 6.2 ppm for lead, 6.3-10.8 ppm for copper, 0.10-0.15 ppm for cadmium, 0.48-1.4 ppm for chromium, 0.09-0.70 ppm for nickel, ND-0.51 ppm for cobalt, ND-2.1 ppm for selenium, and ND-23 ppm for rubidium.

Discussion

These analyses were performed in conjunction with feeding studies in which differences in growth and survival of several marine organisms were observed. Although growth, which would include the effects of naupliar size on acceptance and energy expended in feeding, may

be an important indicator of overall food value, we feel that survival is a more important factor in rating *Artemia* sources as potential live food for animals during the early stages of their development and that survival would also be a more definitive indicator of pollutant-induced stress.

In feeding experiments with two species of crab (Johns *et al.*, 1980) those fed nauplii from Utah and San Pablo Bay did not survive beyond the megalopa stage. With winter flounder, which also undergoes a metamorphosis, survival was also low on Utah and San Pablo nauplii (Klein-MacPhee *et al.*, 1980), while San Pablo Bay nauplii provided the poorest survival in feeding experiments with Atlantic silversides, which does not undergo metamorphosis (Beck *et al.*, 1980).

Since the amino acids (Seidel *et al.*, 1980) and carotenoid contents (Soejima *et al.*, 1980) of all *Artemia* compared in this study were similar, we have scrutinized the lipid, chlorinated hydrocarbon and metal data for possible patterns which might explain the differences in survival of the test organisms fed the various *Artemia* nauplii. Schauer *et al.* (1980) found some differences in total lipid, total fatty acid and 16-carbon fatty acids, but the most striking feature of their data is the high 18:3ω3 and low 20:5ω3 contents of the Utah and San Pablo *Artemia*. Complicating any simple explanation based on fatty acid distribution was the fact that both lots of 1975 San Francisco Bay *Artemia*, one with a low 20:5ω3 content, did promote good growth and survival through the larval development of the mud crab (Johns *et al.*, 1978).

Our analyses clearly show that there are differences in chlorinated hydrocarbons between *Artemia* samples from different locations. The samples from Brazil and Australia were lowest in all types of chlorinated hydrocarbons, while those from Italy and San Pablo Bay were highest in several compounds.

In looking for residues that might explain the poorer performance of San Pablo Bay nauplii, chlordane, dieldrin, DDTs and PCBs, especially the more highly chlorinated 1260 type, would be suspect. The Italian nauplii, however, which did support good growth and survival, contained much higher levels of DDT and thus DDT does not appear to be the causative factor. Moreover, the Utah nauplii, which did not support survival of flounder and crabs through metamorphosis, contained less chlordane, DDT, and PCB than the Italian strain. Of the chlorinated hydrocarbons, therefore, only dieldrin at low levels is common to both Utah and San Pablo nauplii. Since dieldrin LC_{50}'s for aquatic organisms are generally similar to, or higher than those for DDT, it seems unlikely that dieldrin alone could have the effects noted, unless a dieldrin sensitive mechanism is operating at metamorphosis.

The bioaccumulation data for silversides (Table III) show that chlordanes, dieldrin, DDTs and PCBs were accumulated by the fish and would point to chlordane, dieldrin high molecular weight PCBs as potential factors in the lower survival under the San Pablo regime. However, the mud crab accumulations (Table IV) are lower and offer no explanation for the poor survival with the Utah diet.

The concentrations of various metals found in these *Artemia* nauplii fall in the range of concentrations for marine organisms summarized by Byran (1976). If dietary toxicities follow the pattern of lethal concentrations in water, one might expect copper, lead, and cadmium to be the most harmful of the metals determined. However, none of the metal concentrations in any nauplii appear to be exceedingly high and there does not appear to be any metal common to Utah and San Pablo which is dramatically higher than that found in other *Artemia*. The

Utah nauplii do contain slightly more lead, nickel, selenium, and rubidium than other nauplii and the Utah and San Pablo nauplii were slightly lower in iron and slightly higher in copper than the Brazilian or Italian nauplii.

Although LC_{50}'s for chlorinated hydrocarbons and metals have been reported for various freshwater and marine organisms (*e.g.* Eisler, 1970 ; Holden, 1973 ; Mayer *et al.*, 1977) we can find no published values for the toxicities of these compounds introduced by way of the diet to silversides, larval flounder, or mud crab larvae. Eisler (1970) reported LC_{50}'s of 0.4 ppb pp-DDT, 5 ppb dieldrin and 9 ppb γ-BHC for Atlantic silversides, while Epifanio (1971) observed mortality of two crab larvae with 1 ppb dieldrin in seawater. Smith and Cole (1973) subjected adult winter flounder to 2 ppb DDT and 2 ppb dieldrin in water prior to spawning. Mortality of the eggs was related to residue levels in the ova and was higher in the DDT treatment. All flounder larvae, including controls, died at onset of feeding, so residual pesticide effects, if any, could not be assessed.

Variable results have been obtained in bioaccumulation studies where dietary *versus* environmental (water) inputs were compared. Macek *et al.* (1979) conclude that except for DDT, uptake from the diet will be very small compared to bioconcentration from water. Reinert *et al.* (1974), however, found that lake trout over several months accumulated similar levels of pesticide from water containing 0.01 ppb DDT or dieldrin *versus* food containing 2 ppm DDT or dieldrin. When the fish were placed in pesticide-free water, dieldrin was gradually eliminated, but DDT was not. In most comparisons, water concentrations have been much higher than those normally encountered in natural waters. Furthermore, levels of pollutants added or found in dietary studies have been much higher than the levels we found in the *Artemia* nauplii from five geographical locations. Thus it is difficult to predict the possible effects of the relatively small amounts of chlorinated hydrocarbons and metals fed as live *Artemia* in our studies.

Finally, Soejima (personal communication, 1979) isolated chlorophyll from the San Pablo cysts in the course of a study on the carotenoids in *Artemia* (Soejima *et al.*, 1980). This would presumably indicate a higher level of algae in the diet of the San Pablo Bay *Artemia*. Analysis of Brazilian and San Pablo cysts for toxin from dinoflagellates, especially *Gonyaulax sp.*, disclosed no measurable amounts of paralytic shellfish poison (Y. Shimizu, personal communication, 1980).

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